Rat Renal Epinephrine Synthesis

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Abstract

Rats that underwent adrenal demedullation had a 93% decrease in plasma epinephrine (E) levels, but did not decrease their renal E. Even further treatment with 6-hydroxydopamine and reserpine failed to lower renal E levels. Similarly, urine E levels failed to decrease after adrenal demedullation and renal denervation. There is a renal E-synthesizing enzyme that differs from adrenal phenylethanolamine-*N*-methyltransferase (PNMT) in that it is only weakly inhibited by SKF 29661 and can synthesize epinine from dopamine, while adrenal PNMT does so poorly. When an adrenalectomized rat received intravenous [^{3}H]methionine, its urine contained radioactivity that appeared to be [^{3}H]E, with small amounts of [^{3}H]epinine. However, after [^{3}H]methionine was infused in the renal artery, the major product in urine appeared to be [^{3}H]epinine, with a small amount of [^{3}H]E. Adrenal demedullation induced renal E synthesis, but denervation returned the rate of renal E synthesis to control values. The combination of adrenal demedullation, 6-hydroxydopamine, and reserpine treatments increased renal E-forming activity to 350% of control.

We conclude that appreciable portions of renal and urinary E are synthesized in the kidney by an enzyme distinct from PNMT. The enzyme is induced by some treatments that lower E and NE levels.

Introduction

When epinephrine (E)[1] is given to man at doses that have little effect on blood pressure, renal vascular resistance and renin release increase. Sodium, potassium, and chloride excretion into urine and renal blood flow decrease (1, 2). These effects are mediated by E stimulation of α_1, α_2, β_1, and β_2 receptors present in renal tissue (3–5). These receptors mediate renin release, renal vascular tone, and sodium reabsorption. E facilitates neuronal release of NE so it enhances vasoconstriction induced by the firing of renal sympathetic nerves (6). However, when α receptors are blocked, E increases renal blood flow by activating renal β_2 receptors (7).

Stress increases plasma E levels and changes kidney function. The kidney clears ~ 70% of E entering the renal artery (8). Some of this E is taken up by renal sympathetic nerves where it is later released upon neuronal firing (9). Even in unstressed rats adrenal E may modulate kidney function as adrenal demedullation decreases plasma E levels and increases renal α and β receptor number by ~ 25% (10).

E made outside the kidney has important effects on renal function. We find that the kidney itself can synthesize a major fraction of renal and urinary E.

Methods

12 male Sprague-Dawley rats (180–200 g) underwent bilateral adrenal demedullation (Mx) and 7 of these also underwent unilateral renal denervation, later verified by determining the degree of renal NE depletion. Six rats were sham operated.

The five demedullated rats that were not denervated were injected with 6-hydroxydopamine (20 mg/kg i.p.; Sigma Chemical Co., St. Louis, MO) 6 d after Mx. 1 d later these rats were injected with the first of four successive daily doses of reserpine (5 mg/kg i.p.; Sigma Chemical Co.). Rats treated by Mx, 6-hydroxydopamine, and reserpine are referred to as D6R rats. All other rats received vehicle alone. Approximately 24 h after the final injection the Mx rats were anesthetized with pentobarbital (50 mg/kg i.p.) and the ureter of the innervated kidney and the bladder were catheterized. After rinsing the bladder with 1 ml saline, urine was collected from both bladder and ureter. Urine was collected from sham-operated and D6R rats by handling induced urination. After recovery from anesthesia all rats were killed by decapitation, a potent stimulus to adrenal E release. Trunk blood was immediately collected into heparinized tubes and centrifuged at 4°C, and plasma was frozen at −70°C until assay. The kidneys were then removed and each was rapidly weighed and frozen. Before assay each kidney was thawed and homogenized in 1 ml of 0.1 M Tris buffer, pH 7, with 0.1% Triton X-100 by inserting a polytron probe (Brinkmann Instruments, Inc., Westbury, NY) into a test tube packed in ice for 20 s. The homogenate was centrifuged for 10 min at 6,000 *g* and the supernatant was pipetted off. Creatinine levels were measured on diluted urine samples by a colorimetric method (11). Catecholamine levels were measured in duplicate using 50-μl supernate samples according to the radioenzymatic method of Ziegler et al. (12). E-forming activity was assayed radioenzymatically with NE as substrate and [^{3}H]*S*-adenosylmethionine as methyl donor. The [^{3}H]E formed was selectively extracted by alumina adsorption (13).

The inhibitor specificity of the renal E-forming enzyme was determined by assaying kidney and adrenal supernates from five rats in the presence of 10^{-3} M NE substrate and 10^{-4} M SKF 29661 (a phenylethanolamine-*N*-methyltransferase [PNMT] inhibitor). To determine substrate specificity kidney and adrenal supernates from five rats were assayed using both 10^{-4} M NE and 10^{-4} M dopamine (DA).

To determine if the in vitro production of E and epinine by kidney occurs in vivo, we infused rats with L-[methyl ^{3}H] methionine (75 Ci/mmol; Amersham Corp., Arlington Heights, IL). Methionine is the precursor to *S*-adenosylmethionine.

A male Sprague-Dawley rat was anesthetized with 50 mg/kg pentobarbital and adrenalectomized. The bladder was catheterized and an intravenous infusion of 80 μCi [^{3}H]methionine was given over 45 min.

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1. *Abbreviations used in this paper:* ANOVA, analysis of variance; DA, dopamine; D6R, demedullation, 6OHDA, and reserpine; E, epinephrine; Mx, adrenal medullectomized; NMT, *N*-methyltransferase; PNMT, phenylethanolamine-*N*-methyltransferase.

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After the infusion was stopped, urine produced over the following 30 min was collected and analyzed.

A second rat was prepared similarly, but the tip of the infusion catheter was placed in the aorta at the origin of the renal arteries. The abdominal aorta was then ligated distal to the renal arteries and an infusion of 250 μCi of [^{3}H]methionine given over 45 min. After the infusion was stopped, urine produced over the following 30 min was collected and analyzed.

Urine was analyzed for [^{3}H]E and [^{3}H]epinine by a technique adapted from our radioenzymatic assay for catecholamines (12). In brief, 100 μl of urine was mixed with the enzyme catechol-*O*-methyltransferase, *S*-adenosylmethionine, pH 8.3 buffer, $MgCl_2$, and reduced glutathione. After incubation at 25°C for 90 min E was methylated to metanephrine and epinine to *O*-methylepinine. 50 μg of metanephrine and *O*-methylepinine were then added to the incubation mixture along with pH 10 borate buffer. The material was extracted into 5 ml of 3:2 toluene-isoamyl alcohol and back-extracted into 250 μl of 0.1 M acetic acid. After washing with 3:2 toluene-isoamyl alcohol the acetic acid layer was spotted on a silica gel TLC plate. The plate was developed in a 2:16:3 mixture of ethylamine (70%), chloroform, and ethanol. The spots containing metanephrine and *O*-methylepinine were identified under ultraviolet light and scraped into a scintillation vial, and radioactivity was counted.

Data analysis was performed on MR-TOD software (Retriever Data Systems, Seattle, WA).

Results

Plasma E levels were high after decapitation, but Mx and D6R treatment markedly lowered both E and NE levels (Fig. 1). Despite these decreases in circulating E and NE, kidney E levels did not decrease in D6R or Mx rats (Fig. 2). Similarly, urine E levels were reduced much less than plasma E levels in D6R and Mx rats (Fig. 3). The denervated kidney of Mx rats produced urine at a rate of 2.9±8 μl/min, faster than the innervated kidney flow rate of 1±0.2 μl/min ($P < 0.05$). The creatinine content of urine from the denervated kidney (77±13 mg/dl) did not differ from that of the innervated kidney (57±12 mg/dl), nor did the E content of the urine (Fig. 3). The ratio of E to creatinine in urine from the denervated kidney (13.4±9 ng/mg) was not significantly different from the ratio in urine from the innervated kidney (5.8±2.6 ng/mg). The denervated kidney put out a greater volume of urine and more E than the innervated kidney.

In sham-operated rats the kidney had 5.2 pmol/g per h of enzyme activity (Fig. 4). After Mx, E-forming activity was increased to 9.4 pmol/g per h ($P < 0.05$) in the innervated kidney. The denervated kidney had 40% less ($P < 0.05$) E-forming activity than the innervated kidney. In contrast, D6R treatment caused a 350% increase in *N*-methylating activity (Fig. 4).

Kidney E-forming activity was inhibited only 13% by SKF 29661 at a concentration that decreased adrenal PNMT activity by 88% (Table I). The kidney enzyme methylated DA at about two-thirds the rate of NE. In contrast, adrenal PNMT methylated DA only ~ 1% as well as NE.

After an intravenous infusion of [^{3}H]methionine into an adrenalectomized rat, urine collected over a 30-min period contained 645 dpm/ml of radioactivity that chromatographed with E and 53 dpm/ml that chromatographed with epinine. This ratio was reversed after an infusion of [^{3}H]methionine into the renal artery of an adrenalectomized rat. After the intrarenal infusion 50 dpm/ml chromatographed with the E band and 1,084 dpm/ml chromatographed with epinine.

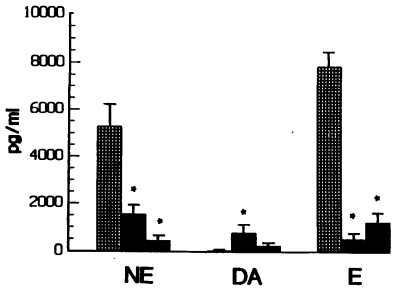

Figure 1. Plasma catecholamine levels from trunk blood collected immediately after decapitation of sham-operated (■), Mx (▨), and D6R rats (■). Each value is the mean±SEM of 5–7 rats. Sham, Mx, and D6R rats differed significantly from each other by analysis of variance (ANOVA) with respect to plasma levels of NE, DA, and E. *$P < 0.05$ vs. sham by Duncan's test.

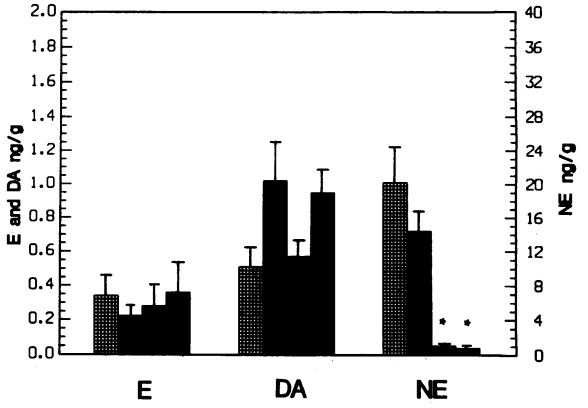

Figure 2. Catecholamine concentrations in renal tissue of sham-operated (■), innervated kidney of Mx (▨), denervated kidney of Mx (■), and D6R rats (■). Each value is the mean±SEM of 5–7 rats. The renal NE levels of the four treatment groups differed significantly from each other by ANOVA ($P < 0.0001$). *$P < 0.05$ vs. sham by Duncan's test.

Discussion

An *N*-methylating enzyme present in rat kidney can synthesize E in vivo. Mx and D6R treatments both decreased plasma E levels to < 7% of control concentrations. However, kidney E levels were essentially unchanged by these treatments and urine E levels were decreased much less than plasma E, so renal tissue appears to synthesize renal and urinary E after Mx. Pendleton et al. (14) found that urinary E was not reduced after Mx in rats. In man, Von Euler et al. (15) reported unchanged urinary E levels after bilateral adrenalectomy. Al-

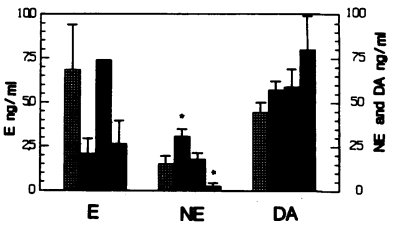

Figure 3. Catecholamine concentrations in urine of sham-operated (■), innervated kidney of Mx (▨), denervated kidney of Mx (■), and D6R rats (■). Each value is the mean±SEM of 4–7 rats. The urine NE levels of the four treatment groups differed significantly from each other by ANOVA ($P < 0.0015$). *$P < 0.05$ vs. sham by Duncan's test.

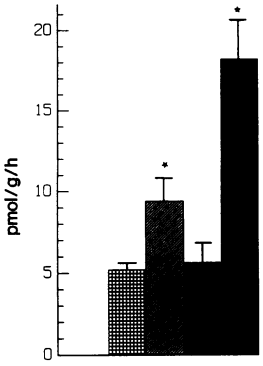

Figure 4. [^{3}H]Epinephrine synthetic rate of kidney homogenates from sham-operated (▩), innervated kidney of Mx (▨), denervated kidney of Mx (▤), and D6R rats (■). Each value is the mean±SEM of 5–7 rats. The [^{3}H]E synthetic rate of the four treatment groups differed significantly from each other by ANOVA ($P < 0.001$). *$P < 0.05$ vs. sham by Duncan's test.

though E can be taken up from blood by the kidney and stored in renal tissues, this effect is relatively minor (16). Axelrod et al. (16) found that < 25% of the E that accumulated in the kidney during an E infusion into cats remained 2 h later, so it is very unlikely that the E that we found in kidney was E that was taken up 11 d earlier, before Mx.

Renal E-forming enzyme differs from adrenal PNMT in both substrate and inhibitor specificity. It methylates DA with a much higher efficiency than PNMT and is not markedly inhibited by 10^{-4} M SKF 29661. We found a similar pattern in other tissues. E-forming activity in the cardiac atrium is primarily PNMT, while that in the ventricle is *N*-methyltransferase (NMT) (13). The localization of PNMT and NMT may have physiologic significance, since an intravenous infusion of [^{3}H]methionine in an adrenalectomized rat produced chiefly [^{3}H]E, while an intrarenal infusion produced primarily [^{3}H]-epinine. Renal homogenates produce more E than epinine when incubated with equal concentrations of NE and DA (Table I), while in vivo the kidney appeared to synthesize more [^{3}H]epinine than [^{3}H]E. This may be related to the high levels of DA in urine (Fig. 3). Renal NE is present primarily in vesicles of sympathetic nerves, but renal NMT is primarily nonneuronal (Fig. 4). Renal DA is synthesized in the kidney from decarboxylation of dihydroxyphenylalanine. It appears that this DA has access to renal NMT since the kidney excretes epinine. It is also possible that the [^{3}H]methionine we infused was converted to [^{3}H]*S*-adenosylmethionine more effectively in renal tissue well supplied with DA.

The activity of renal E-forming enzyme was induced by both Mx and D6R. Ricordi et al. (17) found that extraadrenal E secretion increased for 4 wk after Mx. The induction of extraadrenal E-forming activity such as we found in the kidney could be a source of this extraadrenal E. Since E-forming activity was induced after Mx we cannot give a precise estimate of how much renal E is derived from the adrenal and how much comes from the kidney. Since surgically denervated rats maintained tissue E levels despite loss of the neuronal *N*-methylating enzyme, the nonneuronal enzyme may be a major source of kidney E. This agrees with the finding that most kidney E is nonneuronal (18). In the rat, Pendleton et al. (14, 19) found that chronic treatment with the PNMT inhibitors SKF 7698 for 1–5 d, or SKF 64139 for 3 d increased urinary E excretion. We have found that SKF 7698 and SKF 64139 inhibit extraadrenal E-forming activity much less than adrenal PNMT (unpublished observations). Thus, much of the urinary E Pendleton et al. (14, 19) found may have been synthesized by the renal NMT.

PNMT and NMT are intracellular enzymes that transfer a methyl group from *S*-adenosylmethionine to the amine of catecholamines. *S*-Adenosylmethionine is synthesized in cells from the amino acid methionine. We administered intravenous [^{3}H]methionine radiolabeled on the methyl group so that cells could synthesize *S*-adenosylmethionine capable of transferring a [^{3}H]methyl group to catecholamines. After intravenous administration of [^{3}H]methionine, the highest level of radioactivity extracted with E. After administration into the renal arteries, the greatest amount of radioactivity extracted with epinine. This is in accord with our observations that several organs in the body, such as cardiac atrium and brain, contain PNMT and synthesize E (13). The kidney, however, contains predominantly NMT, which can synthesize E and epinine. Epinine stimulates DA and β-adrenergic receptors; both types of receptors are present in the kidneys (20).

Rat kidneys can synthesize E and epinine in vitro with an enzyme that is inducible and is present in neuronal and nonneuronal tissue. After an in vivo infusion of [^{3}H]methionine the urine contained material that cochromatographed with E and epinine after an extensive procedure of enzymatic methylation, solvent extraction, and TLC. The kidney apparently synthesizes both E and epinine and contains receptors for these catecholamines that alter renin release, renal blood flow, and electrolyte excretion. The role of these locally produced catecholamines in renal physiology is potentially important but remains to be elucidated.

Table I. Effect of PNMT Inhibition and Substrate Beta Hydroxylation on the Rate of N-Methylation by Adrenal and Kidney Homogenates

Tissue	% Inhibition by SKF 29661*	Epinine synthesis rate (% of E synthesis rate)*
Adrenal	88.4±0.6	0.8±0.4
Kidney	12.6±6.5‡	63±8‡

* Values are means±SEM of activity of tissue homogenates from five rats. Inhibition studies were carried out with 10^{-3} M NE and 10^{-4} M SKF 29661.
‡ $P < 0.001$ vs. adrenal.

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